Early enrichment of ESR1 mutations and the impact on gene expression in primary breast cancer treated with aromatase inhibitors in the pre-surgical setting

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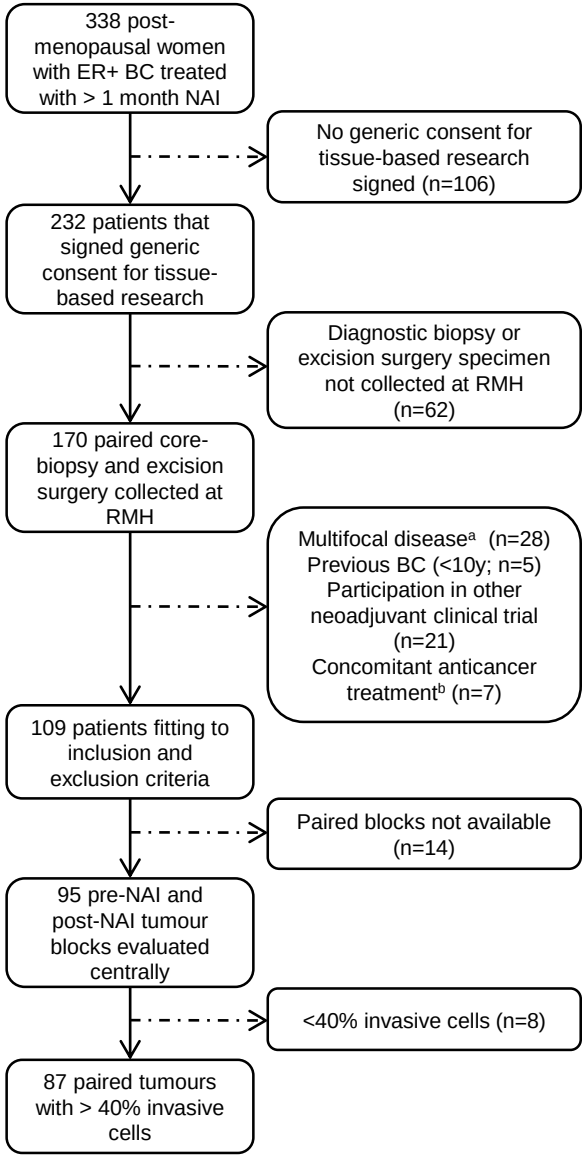
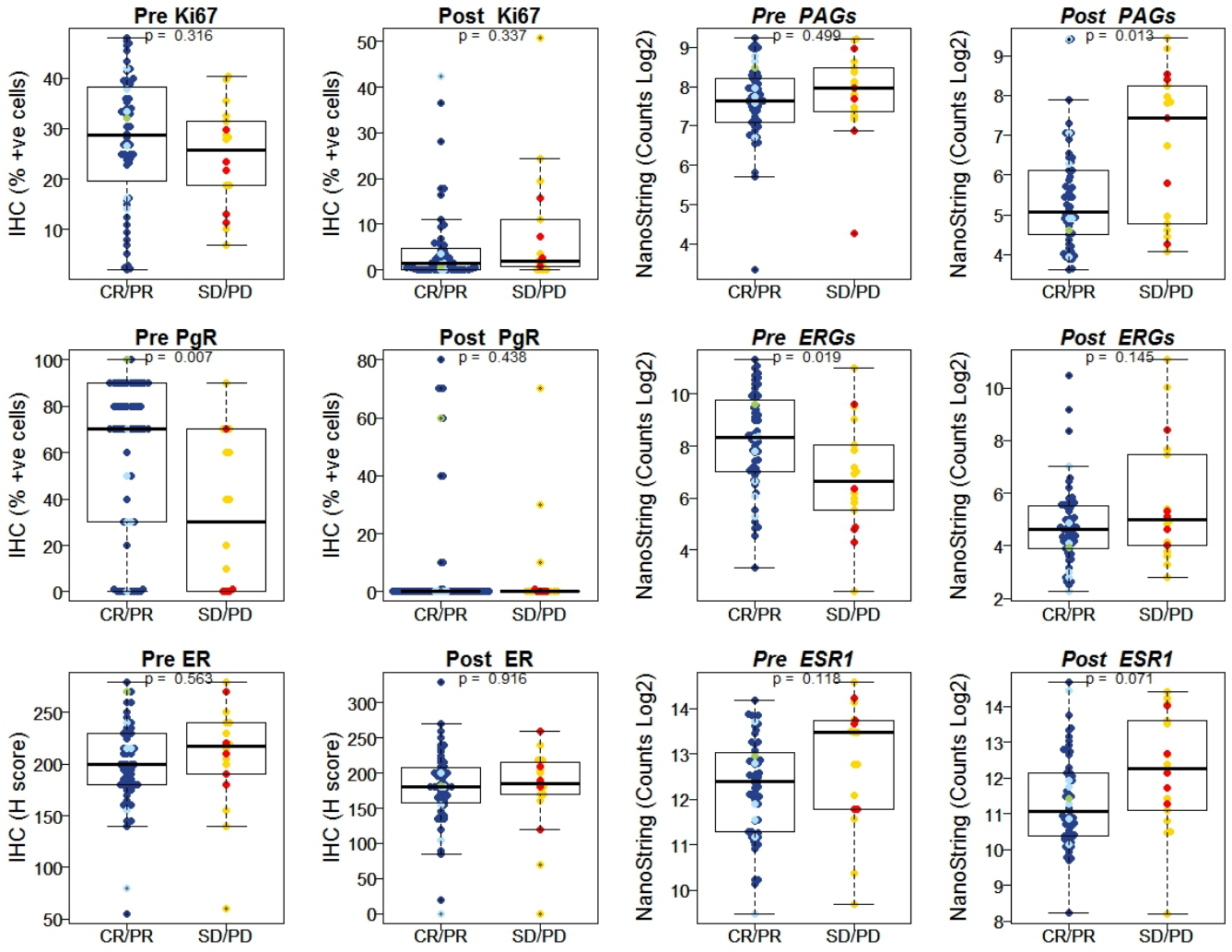
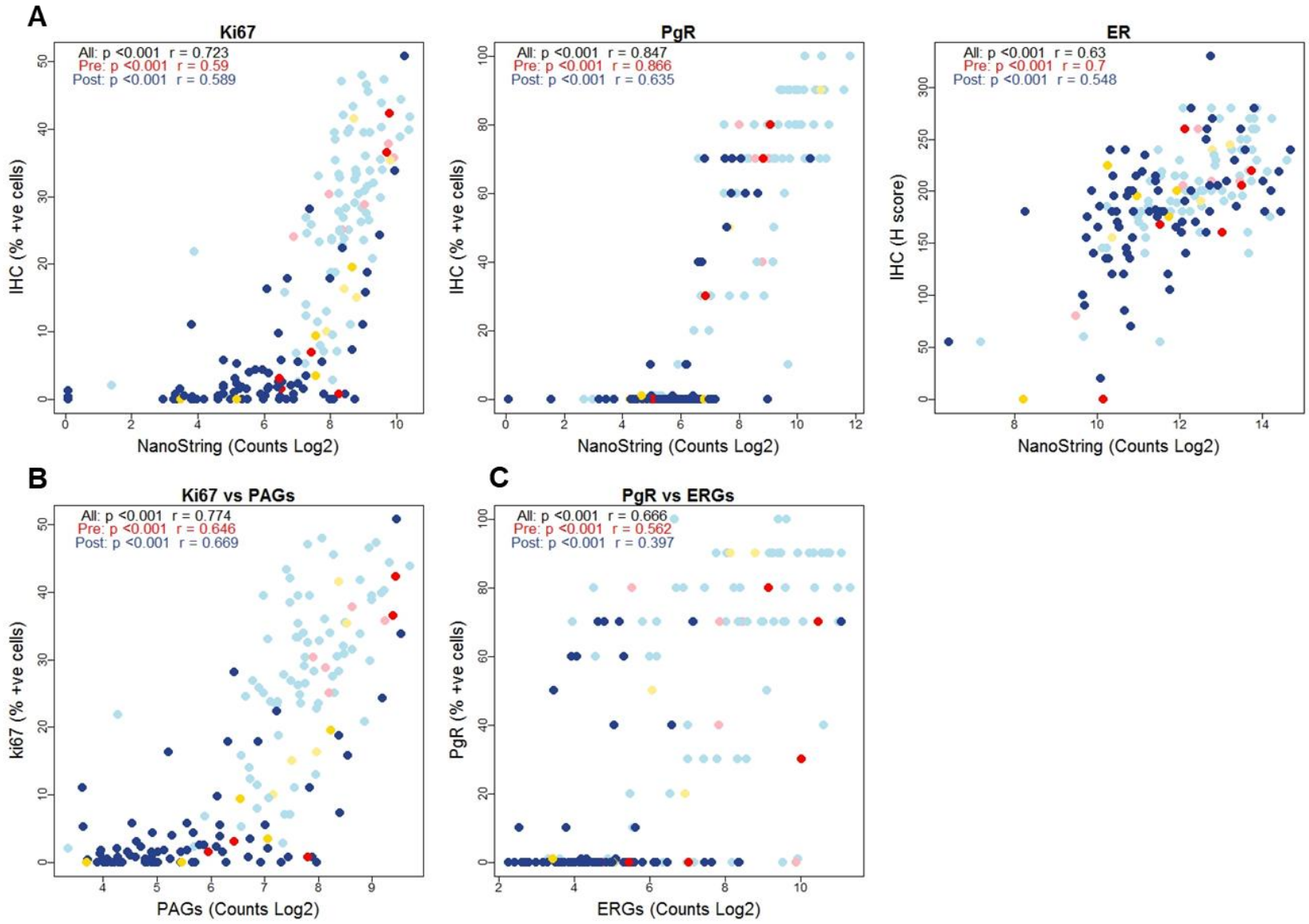
Supplementary figures



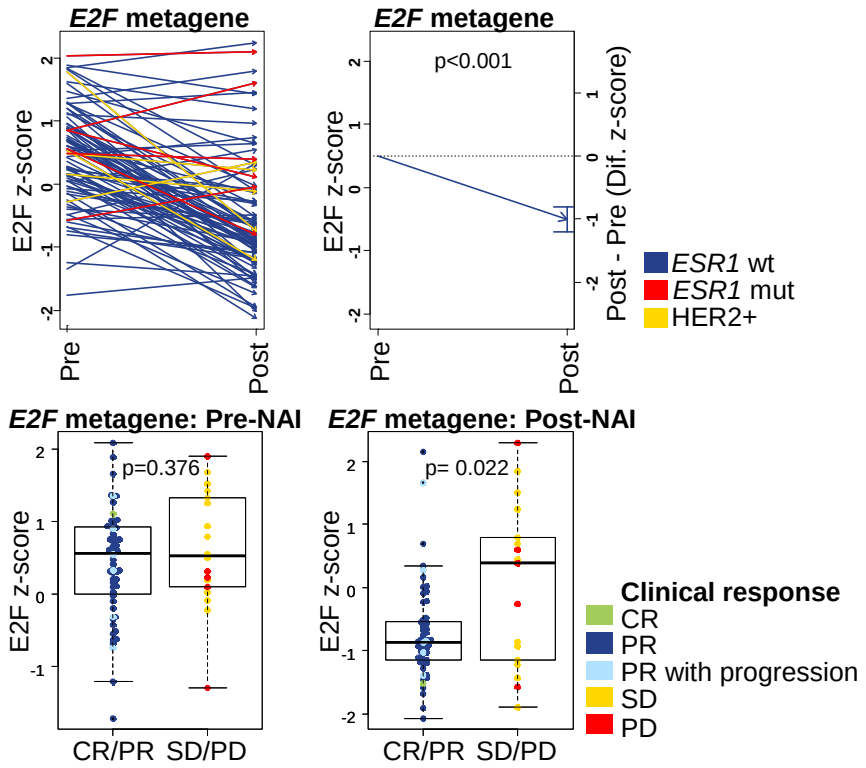
Supplementary Fig. S1. Consort diagram. [a]Multifocal disease confirmed in histopathology analysis. [b]Concomitant anticancer treatments included chemotherapy, biologic response modifiers, endocrine therapy (including steroids) and radiotherapy.



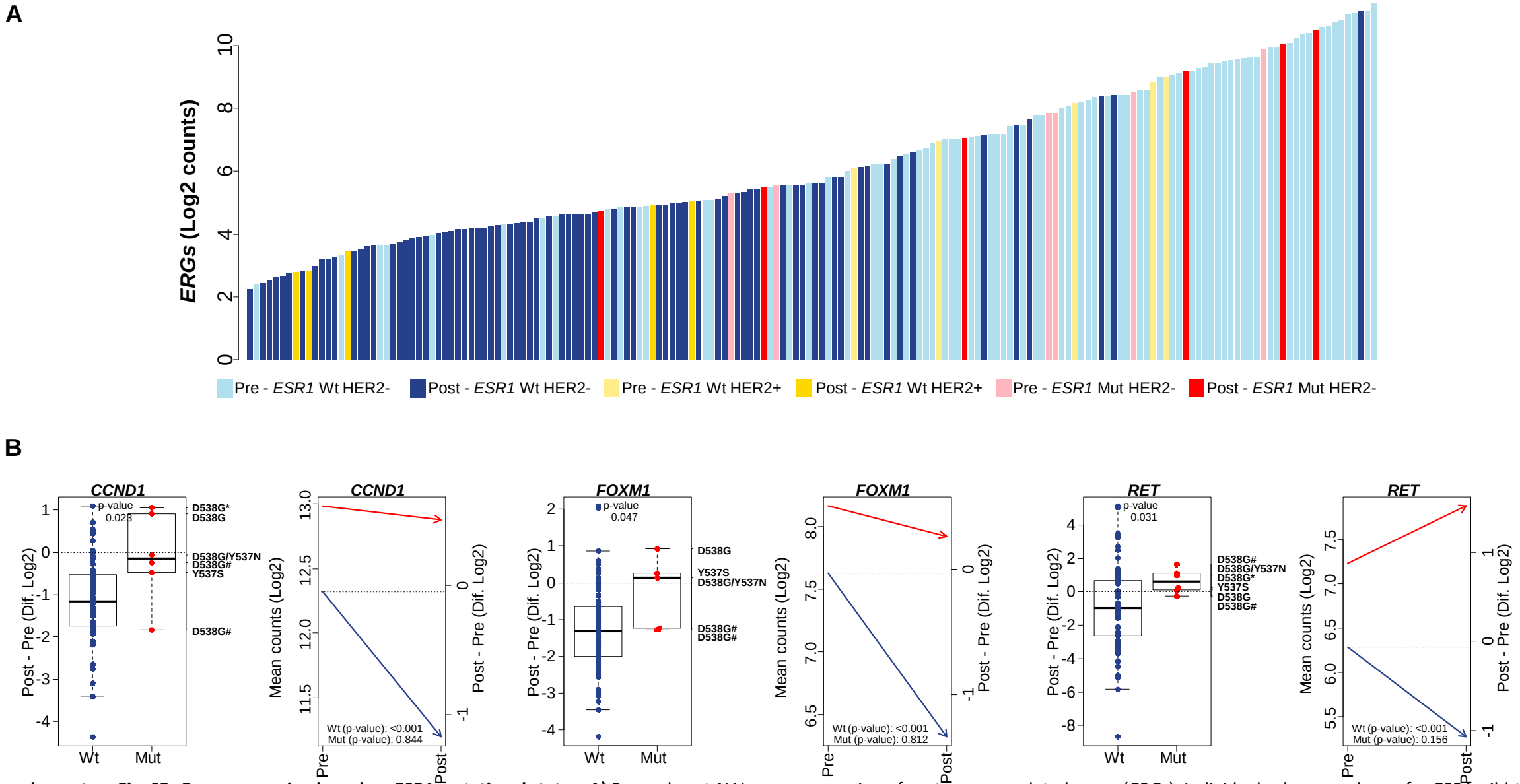
Supplementary Fig. S2. Pre- and post-NAI expression of proliferation markers (Ki67 and proliferation metagene), *ESR1*/ER and *ERGs* based on clinical response stratification. *PAGs*: mean of 11 proliferation genes in the PAM50 gene set; *ERGs*: oestrogen-regulated genes – mean of *TFF1*, *GREB1*, *PDZK1* and *PGR.* CR: complete response to therapy (green); PR: partial response (blue); SD: stable disease (yellow); PD: progressive disease (red). Light blue dots mark cases with PR that showed clinical signs of progression disease (>20% increase of the tumour volume in relation to the previous ultrasound). P-values based on T-test are shown.



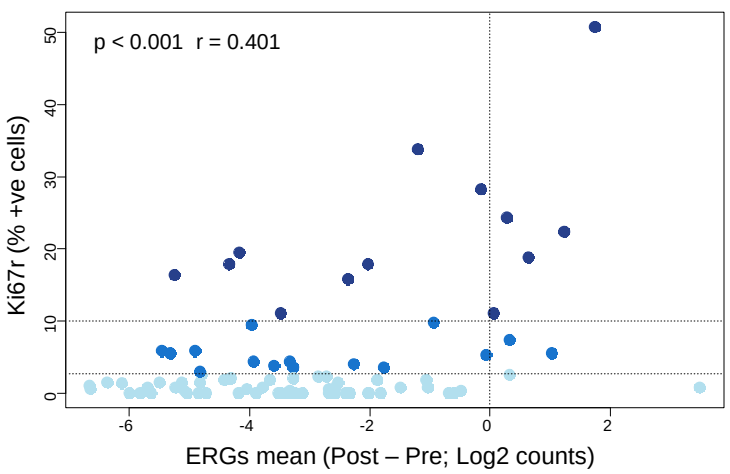
Supplementary Fig. S3. Correlation between protein and gene expression. A) Ki67, PgR and ER expression measured by NanoString™ technology and IHC. **B)** Correlation between Ki67 protein expression and *PAGs*. **C)** Correlation between PgR protein ad *ERGs*. Individual blue dots mark *ESR1* wild-type HER2- tumours, yellow dot *ESR1* wild-type HER2+ tumours and red dots *ESR1* mutant HER2- tumours. Light colours: pre-NAI values; Dark colours: post-NAI values. P-values and coefficient of correlation (r) based on Pearson correlation test are shown. *PAGs*: mean of 11 proliferation genes in the PAM50 gene set; *ERGs*: oestrogen-regulated genes – mean of *TFF1*, *GREB1*, *PDZK1* and *PGR.*

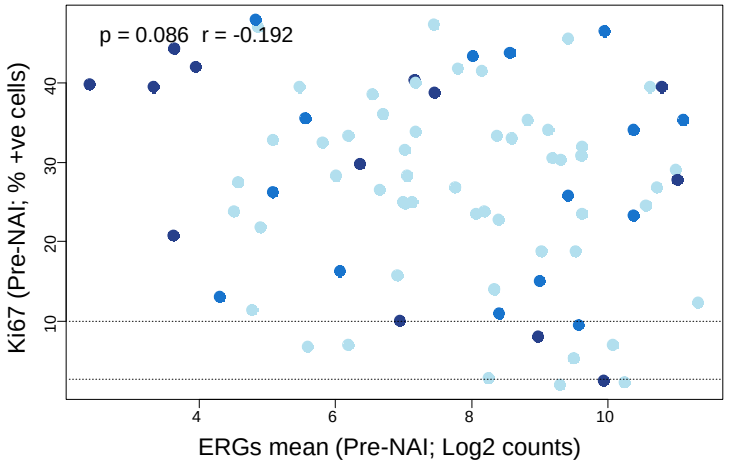


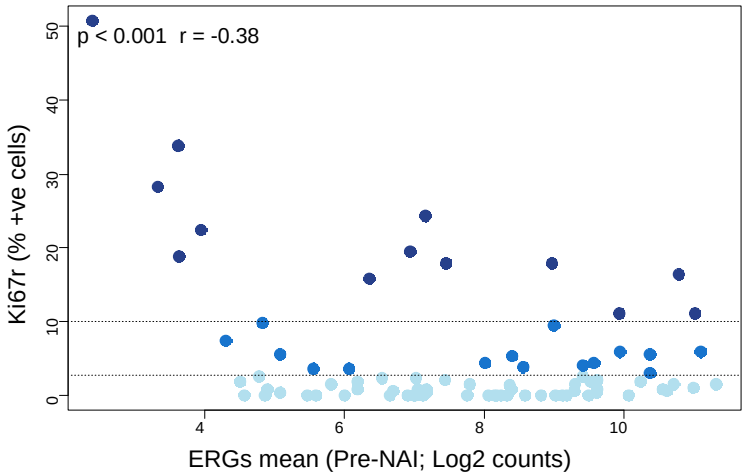
Supplementary Fig. S4. E2F activation metagene in NAI-therapy. Overall inhibition of *E2F* activation metagene with NAI treatment and higher post-NAI expression of this signature in patients with stable disease / progressive disease (SD/PD) in comparison with complete or partial response (CR/PR) in both pre- and post-NAI samples based on clinical response stratification. Arrow graphs represent the individual expression (left) and the mean expression with the 95% confidence interval of the mean difference (right) in pre-NAI and post-NAI samples. P-values based on T-test (box plots) or paired T-test (arrow plots) are shown.



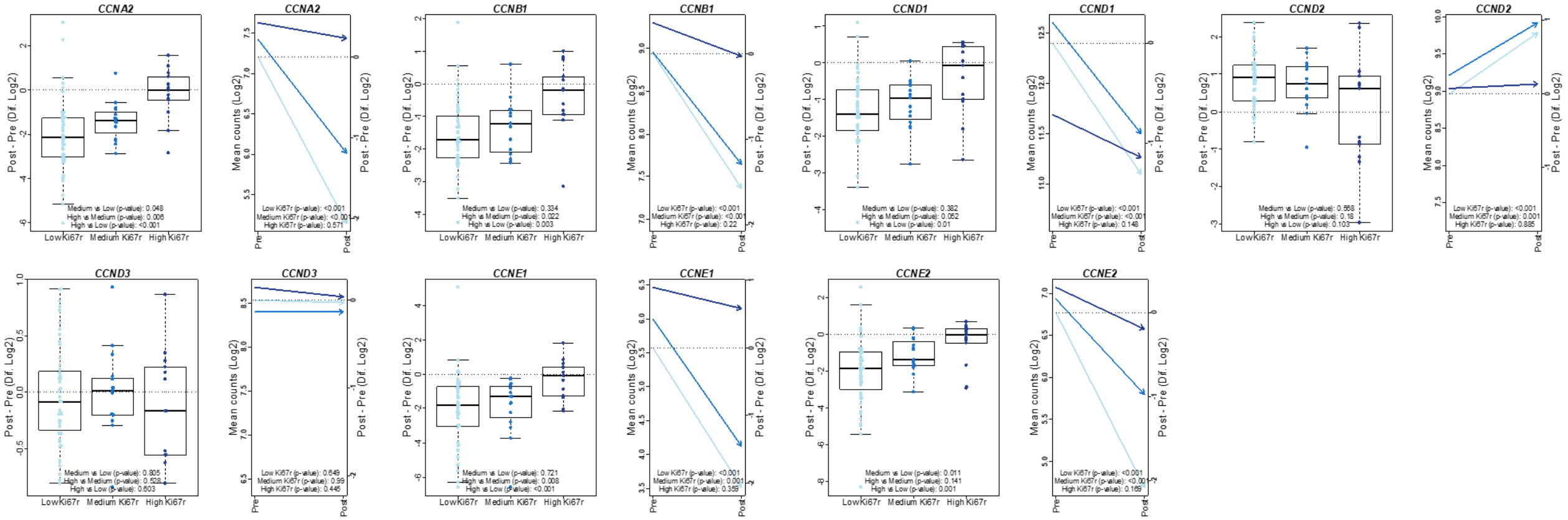
Supplementary Fig. S5. Gene expression based on *ESR1* mutational status. A) Pre and post-NAI mean expression of oestrogen-regulated genes (*ERGs*). Individual values are shown for *ESR1* wild-type HER2- tumours (blue bars), *ESR1* wild-type HER2+ tumours (yellow bars) and *ESR1* mutant HER2- tumours (red bars). Light colours: pre-NAI values; Dark colours: post-NAI values. **B)** *CCND1*, *RET* and *FOXM1* expression in *ESR1* wild-type (blue dots and arrows) and mutant tumours (red dots and arrows). Less inhibition of these biomarkers was detected in *ESR1* mutant tumours. Box plot graphs represent the expression difference (Post-NAI – Pre-NAI) with individual values also shown. Arrow graphs (right) represent the mean expression of each group in pre-NAI and post-NAI samples. P-values based on Mann-Whitney test (box plots) or Wilcoxon (arrow plots) are shown. *ERGs*: oestrogen-regulated genes – mean of *TFF1*, *GREB1*, *PDZK1* and *PGR.* Wt: *ESR1* wild-type tumours; Mut: tumours harbouring *ESR1* mutation. *ESR1* mutation type are highlighted.







Supplementary Fig. S6. Correlation between *ERGs* and Ki67 expression in *ESR1*^{Wt} tumours. Light blue: low residual Ki67 (% of +ve cells ≤2.7%, n=53). Bright blue: medium level of residual Ki67 (>2.7% & ≤10%, n=15). Dark blue: high residual Ki67 (≥10%, n=13). P-values and coefficient of correlation (r) based on Pearson correlation test are shown.



Supplementary Fig. S7. Change in cyclins expression in *ESR1*^{Wt} tumours classified based on Ki67r. Box plots represent on-treatment change. Arrow graphs (right) represent the mean expression of each group in pre- and post-NAI samples. Light blue: low residual Ki67 (% of +ve cells ≤2.7%, n=53). Bright blue: medium level of residual Ki67 (>2.7% & ≤10%, n=15). Dark blue: high residual Ki67 (≥10%, n=13). P-values and coefficient of correlation (r) based on Pearson correlation test are shown. P-values based on T-test (box plots) or paired T-test (arrow plots) are shown. Ki67r: residual Ki67 (post-neoadjuvant AI therapy).